Permethylation

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Reagents:
- Sodium Hydroxide Solution, 50% w/w
- Methanol (MeOH)
- Dimethyl sulfoxide (DMSO)
- Iodomethane (MeI)
- Dichloromethane (DCM)
- HPLC Water
- Acetic acid
- n-propanol (1-PrOH)

Equipment/Apparatuses:
- N_2 evaporator
- Sep-Pak Vac 1cc (100mg) tC18 (Waters, Cat# WAT036820)
- Glass threaded culture tube (round bottom)
- Phenolic cap with PTFE-Faced rubber liner
- Glass pipette
- Plastic pipette
- Glass syringe
- Centrifuge

Procedures:

The following procedures describe small-scale permethylation reactions prior to MS analysis. In order to achieve robust permethylation, NaOH slurry must be freshly prepared and all glassware must be scrupulously cleaned. The protocol is an SOP permethylation procedure used at CCRC, which is a slightly modified procedure of the method of Anumula and Taylor (1992)[1]
Also, the video instruction is available at Nix et.al (2014) [2]

1. [Base Preparation for Permethylation]

1.1) To prepare the base reagent for permethylation, add 400 µl of 50% NaOH to a clean glass screw-top tube (13 x 100 mm). Add 800 µl of anhydrous MeOH and vortex.

1.2) Add 4 ml of anhydrous dimethyl sulfoxide (DMSO) and vortex. A white precipitate will be generated.

1.3) Centrifuge at 2,500 rpm (600 x *g*) for 1 min to pellet the basic slurry. Pipette off supernatant and the non-pelleted white material.

1.4) Repeat steps 1.2 and 1.3 a minimum of three more times or until no white precipitate forms.

1.5) Dissolve the pelleted base in 3 ml anhydrous DMSO and gently mix by pipetting up-and-down with a clean glass pasteur pipette.

Note:
The base should be used in the same day for the following permethylation reaction.

2. [Permethylation of Glycolipid]

2.1) This procedure is optimized for small amount of glycolipid (up to 10ug). To avoid underpermethylation reaction, the amount of glycolipid needs to be estimated by TLC analysis prior to MS analysis.

2.2) Add 100 µl of anhydrous DMSO to the dried sample and vortex to dissolve.

2.3) Add 300 µl of the resuspended base slurry to the sample and immediately add 100 µl of iodomethane (MeI). Seal tube with Teflon-lined cap and vigorously mix for 5 min by vortex.

2.3) To stop the permethylation reaction, add 2 ml of 5% AcOH on ice and vortex.

2.4) Add 2 ml of dichloromethane (DCM) and vortex. Centrifuge at 2,500 rpm (600 x *g*) for 1 min at room temperature to separate the aqueous and organic phases.

2.5) Remove the top layer (aqueous phase) and discard.

2.6) Add 2 ml of H_2O to the organic phase and vortex. Centrifuge at 2,500 rpm (600 x *g*) for 1 min to separate the aqueous and organic phases.

2.7) Repeat steps 2.6 and 2.5 three more times.

2.8) Remove the final top layer (aqueous phase) as much as possible from the bottom layer (organic phase) and transfer the bottom layer (organic phase) into a new glass tube using a clean pasteur pipette.

2.9) Dry the organic phase under N_2 stream at 40 °C.

2.10) Store the sample at -20 °C until use.

Note:

- Permethylated glycolipid can be further desalted by passing through C18 column prior to MALDI-TOF-MS analysis to obtain stronger signal intensity.

- The desalting step is not required for NSI-MS analysis.

3. [C18 Clean-Up of Permethylated Glycolipid] (Optional)

3.1) Equilibrate a C18 cartridge column with three volumes of MeOH and five volumes of 5% AcOH.

3.2) Reconstitute the permethylated glycolipid in 50% MeOH-water and load onto the column.

3.3) Wash the column with 10 ml of H_2O for desalting.

3.4) Elute the permethylated glycolipid into a new glass tube with 2 ml of n-PrOH.

3.5) Dry eluates under nitrogen stream at 40 °C.

References

1. Anumula, K.R. and P.B. Taylor, *A comprehensive procedure for preparation of partially methylated alditol acetates from glycoprotein carbohydrates*. Anal Biochem, 1992. **203**(1): p. 101-8.
2. Nix, D.B., et al., *Improved in-gel reductive beta-elimination for comprehensive O-linked and sulfo-glycomics by mass spectrometry.* J Vis Exp, 2014(93): p. e51840.

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